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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Fiorentini S, Messali S, Zani A, et al. First detection of SARS-CoV-2 spike protein N501 mutation in Italy in August, 2021. *Lancet Infect Dis* 2021; published online Jan 12. http://dx.doi.org/10.1016/S1473-3099(21)00007-4.

A

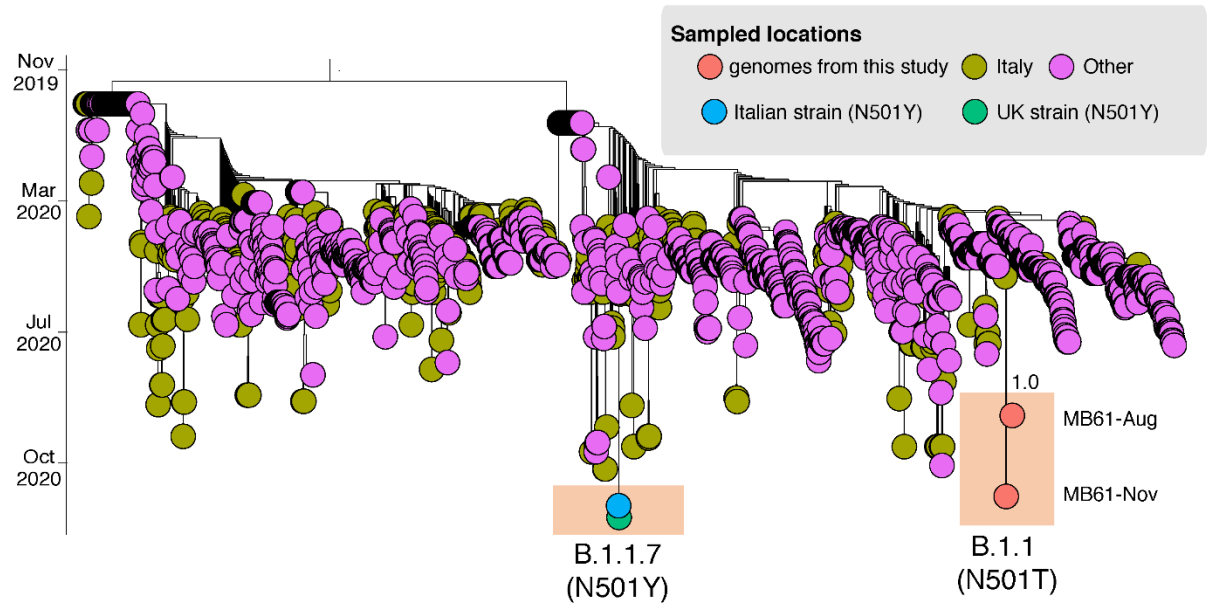
Mutational patten MB61-Aug

Gene	Nucleotide	Amino acid
ORF1a	G5180	D1639N
	C14408T	P4715L
	C21005T	A6914V
Spike	A22108C	K182N
	22286-22288 del CTT	L242 del
	C23039A	Q493K
	A23064C	**N501T**
	A23403G	D614G
N	G28881A e G28882A	R203K
	G28883C	G204R

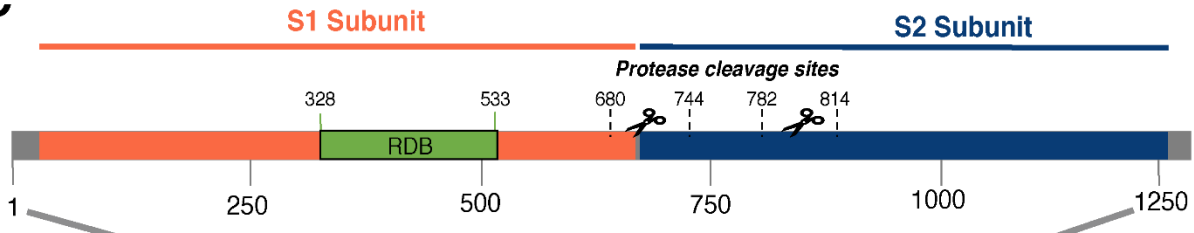
Mutational patten MB61-Nov

Gene	Nucleotide	Amino acid
ORF1a	G5180	D1639N
	C14408T	P4715L
	C17245T	R5661C
	G18883A	V6207I
Spike	C21005T	A6914V
	A22108C	K182N
	22286-22288 del CTT	L242 del
	C23039A	Q493K
	A23064C	**N501T**
	A23403G	D614G
ORF3a	C25714T	L108F
N	G28881A e G28882A	R203K
	G28883C	G204R

B



C



Position	Reference yp_0097243980	MB61	EPI_ISL_728343 EPI_ISL_717978
69-70	HV	HV	del
144	Y	Y	del
182	K	N	K
241	L	del	L
493	Q	K	Q
501	**N**	**T**	**Y**
571	A	A	D
614	D	G	G
681	P	P	H
706	T	T	I
982	S	S	A
1118	D	D	H

Figure: **Sequence analyses of MB61 SARS-CoV-2 variants**

(A) Mutational pattern of the two isolates MB61-Aug and MB61-Nov, which were obtained from the same patient 4 months apart. Highlighted are the amino acid mutations that emerged along viral persistence. (B) Time-scaled maximum likelihood tree including MB61-Aug and MB61-Nov plus 2137 sequences representative of the global SARS-CoV-2 epidemic. MB61 variants are highlighted with red circles. The EPI_ISL_717978 and EPI_ISL_728343 strains, carrying the mutation N501Y in the spike RBD domain, are also highlighted with blue and green circles, respectively. Colours indicate geographical location of sampling. Support for branching structure is shown by bootstrap values at nodes. (C) Amino acid changes in the spike region of the genomes from this study plus the reference yp_0097243980, the EPI_ISL_728343, and the EPI_ISL_717978. Bold line indicates the amino acid 501 position. Red letter shows the Asparagine (N) to Threonine (T) mutation that occurred in MB61 variants.

The one letter amino acid code has been used; del indicates the presence of an amino acid deletion(s).